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SEXUAL DIMORPHISM IN CUNNINGHAMELLA

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(WITH ONE FIGURE)

Introduction

HETEROTHALLIC AND HOMOTHALLIC FORMS

In 1904 (1) it was shown that the mucors can be classified into two main groups according to their ability to produce zygospores from the sowing of a single spore. Species which are able to form sexual spores by the conjugation of branches from the same plant were called homothallic, since the mycelia appeared to be sexually alike; those which were able to form sexual spores only by the interaction of different plants were called heterothallic, since the mycelia taking part in conjugation appeared to be sexually different. The terms homothallic and heterothallic were used instead of hermaphroditic and dioecious because at the time they were first suggested our knowledge of sexuality in the mucors did not seem to warrant unreservedly accepting the idea of a strict sexual dimorphism in these forms, although such a dimorphism was strongly indicated by the interaction which had just been discovered between plus and minus races.

In later publications (5, 6) the desirability was pointed out of extending the use of the terms homothallic and heterothallic to signify the type of sexual differentiation in all gametophytes, in contrast with the terms homophytic and heterophytic suggested for sporophytes. It was not expected that these terms would supplant the more familiar words hermaphroditic and dioecious. They may be found useful in bringing about greater accuracy in sexual terminology, and in emphasizing the inconsistency of calling a form like *Marchantia* dioecious and a form like the common lily hermaphroditic, when the two belong to the same sexual type.

Evidence for sex intergrades in heterothallic species

It is well known that sex intergrades are relatively frequent in sporophytic plants like the willow and hemp, which are commonly classified as dioecious. Similar sexual abnormalities, therefore, have been expected and sought for in heterothallic mucors ever since heterothallism was discovered in these forms in 1903. The fact that no race of a heterothallic mucor[1] has been found by the writers which, if it gave any sexual reaction at all, behaved otherwise than as a plus *or* a minus, indicates that sex intergrades in these forms are at best extremely rare. It is true that a number of investigators have reported findings which they have interpreted as opposed to the existence of a strict sexual dimorphism in the bread molds and related forms. Despite the fact that their conclusions were in harmony with our expectations based upon the condition in higher forms, the instances of supposed sex intergrades in heterothallic species are either isolated observations of two conjugating filaments which seem to originate from the same hypha, or have been supported by experimental evidence open to criticism. The inadequacy of the evidence has been pointed out in earlier publications (4, 7, 8), but it seems appropriate to mention two examples more or less typical of their kind. The first case is cited by Miss McCormick (15), and is illustrated by a figure showing a partially matured zygospore with the suspensor on one side arising from a filament which curves over and connects with the filament from the suspensor on the other side. In reply to an inquiry in regard to this zygospore Miss McCormick has written that after the

[1] The peculiar homothallic mycelium produced by regeneration of the germ tube and at times produced from the germination of spores in the germ sporangium in *Phycomyces* (3) is a temporary condition only, and apparently should more properly be considered a mixochimera of plus and minus protoplasm, as Burgeff (13) suggests, than as a sexually constant race.

drawings were made the homothallic strand was lost in an attempt to make a permanent mount of the fresh material. So far as we are aware, such a condition as Miss McCormick figures has not been described elsewhere for *Rhizopus* since 1903, when heterothallism was first discovered in the mucors. Inasmuch as an enormous number of zygospores of *Rhizopus* must have been observed more or less closely during this time, since it is a common type for laboratory study (Miss McCormick herself [16] reports having examined over 2000 in her cytological investigations in this species), it appears reasonable that in an isolated instance of this kind, the filaments from the two sides of the zygospore which appeared to be connected may in fact have been separate, but the place of separation obscured by overlying hyphae. That it is unsafe to judge of the thallic condition of a species from the hyphal connections is shown by experience with a class of students who were studying *Rhizopus* shortly after heterothallism had been discovered in other mucors, but before it had been demonstrated for this species. They were asked to find cases in which both the suspensors originated from branches of the same filament. A number of cases were found in which the two suspensors actually seemed to be connected, but in every instance there were one or more overlying filaments which would render the condition open to doubt by a critical observer.

The second case is a paper by Namyslowski (17), in which he throws doubts upon heterothallism in the whole group of the mucors. His experimental evidence comes from isolating single spores of *Rhizopus* and sowing the resulting mycelia on bread. The appearance of zygospores in fourteen out of forty-six such single spore cultures led him to conclude that his *Rhizopus* was homothallic. As has already been pointed out (7), the facts that six of these cultures were destroyed by bacteria and that thirteen more were devoid of even sporangia and hence probably also infected with bacteria, rendered it probable, to one familiar with pure culture methods, that the zygospores which Namyslowski obtained in part of his cultures from the sowing of a single spore actually might have arisen through interaction with the opposite sex which had gained access to these cultures through infection. This explanation seemed later confirmed by the isolation of the two sexual strains from zygosporic material which NAMYSLOWSKI kindly sent to one of us. NAMYSLOWSKI (18), however, still believes, upon evidence which we have criticized, that heterothallic species have been shown capable of producing homothallic zygospores.

The two examples given are typical of less careful observations. Although cases of homothallism in heterothallic species on a priori grounds were to be expected, we have never found them ourselves, and could not feel that the reports of them by other investigators could stand critical examination. It reminded one of the reports of the birth of a full black negro baby from pure white parents which from time to time have appeared in literature and been passed on by rumor, but which have in no case been confirmed by students of human heredity.

BURGER ON CUNNINGHAMELLA

The condition outlined was the situation in the early part of 1919, when BURGER announced the finding of hermaphroditic as well as "pseudo-heterothallic" strains in *Cunninghamella* and *Syncephalastrum*. At the same time report came from one of the laboratories of the Department of Agriculture of a strain of *Rhizopus* which would form zygospores with both plus and minus test strains of this species. As to the *Rhizopus*, it was found upon inquiry that this particular strain had died out, and that after all it had not shown the capacity of conjugating with both the opposite sexes. BURGER'S paper on sexuality in *Cunninghamella* (14) presents the most extensive evidence which has yet appeared for sex intergrades in any heterothallic mucors. Although his arguments from the data presented seemed open to some criticism, his publication made the genus *Cunninghamella* the most likely source known for sex intergrades, the investigation of which would have considerable genetic interest. Recently published studies by one of us (11) had shown that races with plus and races with minus tendencies can arise by mutation from a homothallic species, and that such a race may cease to form zygospores and take on the appearance of a heterothallic species. It seemed worth while, therefore, to look for races with homothallic tendencies among heterothallic species, in view of BURGER'S paper. Accordingly, a rather extensive study of the interaction of strains has been made for four species of *Cunninghamella*, the details of which will be given later. Since they offer no support to BURGER'S conception of hermaphroditism in the genus, and since his cultures were allowed to die out before his final results were published, making it impossible for his material[2] to be retested, it is necessary to subject both his experimental technique and conclusions to searching criticism in lieu of other means of judging of the correctness of a statement which runs counter to the experience of most careful workers on the Mucorineae. BURGER'S paper will be considered before discussing the results of this investigation.

BURGER found great irregularity in the sexual behavior of races of *C. bertholletiae*. While some races were consistently either plus or minus in reaction, others appeared to react both as plus and minus with properly chosen test strains. Certain races seemed to form a sexual triangle. His race *A*, for example, would form zygospores with *B*, race *B* would form zygospores with *C*, race *C* would form zygospores with *A*, and the family triangle was complete.

BURGER'S conclusions are based primarily upon tests with twenty-six[3] races of *C. bertholletiae*. Since he says "authentic cultures of *C. bertholletiae* and *C. elegans* were obtained from Holland," and later credits us with having sent the only race of *C. elegans* which he used in his tests, there is little doubt that his race no. 21 of *C. bertholletiae* is identical with the no. 213 which we secured from the Centralstelle, and of which we sent a subculture to the Harvard laboratory with *C. elegans* shortly before BURGER used the strains in his investigations. In addition to these two races, he used the plus and minus strains of *C. echinulata* and of *Mucor V*, which had also been sent by us to the Harvard laboratory. The sexual races of these two species were contrasted with all his twenty-six races of *C. bertholletiae*, but without finding any "imperfect hybridization" reactions. The race of *C. elegans* and six races of *C. bertholletiae* were individually contrasted with the remaining races of a collection consisting of twenty-six races of *C. bertholletiae*, five races of *C. echinulata* (including our plus and minus strains),

[2] Except his race no. 21, which will be discussed later.

[3] In two places in the text (probably through error), his race no. 25 is called *Mucor V* minus.

and plus and minus strains of *Mucor V* and the plus strain of *C. elegans*. The positive and negative results are assembled in a table. The sexual condition seemed to BURGER so hopelessly confused that he was led to the following conclusions contained in his summary:

1. In *Cunninghamella* there does not exist sexual dimorphism.

2. *C. echinulata* plus and minus, or *Mucor V* plus and minus as separated by BLAKESLEE, are unable to form progametes or gametes when contrasted with any one of twenty-six cultures of *C. bertholletiae*.

3. Many of these cultures of *C. bertholletiae* were able to form zygospores when contrasted with certain other cultures of this same species.

4. There exists a selective power in some strains to form zygospores with certain other strains. This condition of pseudoheterothallism cannot be explained at present.

5. There exists a condition in some strains which might be called hermaphroditism.

6. In none of the hermaphroditic strains did branches of the same hyphae conjugate.

7. Zygospores were produced only when two strains were contrasted whose gametes were compatible.

It will be well to examine this summary to see whether the rather sweeping conclusions are warranted from BURGER'S own data, assuming for the moment that these data are not open to criticism. The results of his contrasts are more readily compared if his table be rearranged as shown in table I. The six testers of *C. bertholletiae* are placed at the top, together with the plus race of *C. elegans* and of *Mucor V*, which were also used as testers. On the side, grouped according to sex, are placed the twenty-six races with which the testers were contrasted; *H* stands for imperfect hybridization, *Z* for zygospores. If the latter is inclosed in parenthesis, it indicates the occurrence of zygospores in a contrast where they would not be expected on the basis of a strict sexual dimorphism. No grades are given in table I, since none are presented in the original paper.

The sexual behavior of the twenty-six races of *C. bertholletiae* shown in the table I is not so badly mixed as even BURGER himself was apparently led to believe from his method of analyzing the data. He says: "Nos. 1 and 2 have always remained constantly plus, while nos. 4–6, 12, 15, 18, 19, 22–26 were always minus, nos. 3, 7–11, 13, 14, 16, 17, 20, 21, ... however, have reacted with both the so-called plus and minus strains." This is a curious conclusion, that nos. 1 and 2 are constantly plus because they

TABLE I

Rearrangement of data in Burger's table I: *Cunninghamella bertholletiae*, 26 races (1–26); number of combinations possible, 325; number of combinations made, 135; aberrant combinations, 6; "pseudoheterothallic hermaphroditic races," 3 to 8; Z stands for occurrence of zygospores, H for occurrence of "imperfect hybridization" reaction; parentheses indicate that reaction is aberrant on basis of sexual dimorphism

Races contrasted		9 plus or plus and minus	10 plus	14 plus or plus and minus	34 [*C. elegans* plus]	33 [V plus]	21 minus or plus and minus	7 minus or plus and minus	3 minus or plus and minus
Plus or plus and minus	9...	......	O	O	O	O	Z	Z	Z
Plus	10...	O	......	O	O	O	Z	Z	Z
Plus or plus and minus	14...	O	O	......	O	O	Z	Z	Z
Plus or plus and minus	16...	O	O	(Z)	O	O	Z	Z	Z
Plus	17...	O	O	O	O	O	Z	Z	Z
Plus or plus and minus	20...	(Z)	O	(Z)	O	O	Z	Z	Z
Plus	1...	O	O	O	O	O	Z	O	O
Plus	2...	O	O	O	O	O	Z	O	O
Plus	11...	O	O	O	O	O	O	O	Z
Minus or plus and minus	3...	Z	Z	Z	H	O	(Z)	O	......
Minus or plus and minus	7...	Z	Z	Z	O	O	(Z)	......	O
Minus	8...	Z	Z	Z	H	O	O	O	O
Minus	12...	Z	Z	Z	H	O	O	O	O
Minus or plus and minus	13...	Z	Z	Z	H	O	(Z)	O	O
Minus or plus and minus	21...	Z	Z	Z	H	O	......	(Z)	(Z)
Minus	4...	Z	O	Z	O	O	O	O	O
Minus	5...	Z	O	O	O	O	O	O	O
Minus	6...	Z	O	O	O	O	O	O	O
Minus	15...	Z	O	O	O	O	O	O	O
Minus	18...	O	Z	O	O	O	O	O	O
Minus	19...	O	Z	O	O	O	O	O	O
Minus	22...	O	Z	O	O	O	O	O	O
Minus	23...	O	Z	O	O	O	O	O	O
Minus	24...	O	O	O	H	O	O	O	O
Minus	25...	O	O	O	H	O	O	O	O
Minus	26...	O	O	O	H	O	O	O	O
[V minus]	32...	O	O	O	H	Z	O	O	O

produced zygospores with a hermaphrodite (no. 21) and with no other race; while no. 11 is listed among those which have reacted with both the so-called plus and minus strains when it formed zygospores only with no. 3, which need not be considered other than as a good minus. Burger further believed that there were twelve hermaphrodites, since he lists this number, including no. 11 among those reacting with both plus and minus strains. Referring to table I, it will be seen that in only eight contrasts are zygospores found where under a strict sexual dimorphism they would not be expected. Two of the eight are duplicates, leaving only six different contrasts showing aberrant reactions. It is not necessary, however, to consider more than three races hermaphroditic to account for the aberrant results. These three hermaphrodites may be variously chosen. Race no. 21 has three aberrant reactions, which is the largest number shown by any race. Both races nos. 14 and 20 show two aberrant reactions each, and may be chosen with no. 21 to make up the three hermaphrodites. Since nos. 14 and 20 are both assumed to be hermaphrodites, the reaction between them ought not perhaps to be credited to both of these races. However the credit of aberrancy is adjusted between nos. 14 and 20, race no. 21 remains the one which gives the largest number of aberrant reactions, and therefore of all the twenty-six races it is the one most surely shown by BURGER's data to be a hermaphrodite. This no. 21 is the same as our no. 213, and is the only one of the twenty-six races which it has been possible to reinvestigate. Its sexual behavior will be discussed more fully later.

Conclusion no. 1 of BURGER's summary that in *Cunninghamella* there does not exist sexual dimorphism would seem too sweeping a statement in view of the fact that in *Mucor* and *Absidia*, which are predominantly heterothallic, forms are known, such as *Mucor heterogamus* (which with other similar species has been placed by some workers in the genus *Zygorhynchus*), and *Absidia spinosa*, which are homothallic. Races of two other species of *Cunninghamella* reported upon in the paper under discussion gave no evidence of hermaphroditism, and in consequence the data presented warrant the conclusion at most in reference to a single species. That in this single species three out of twenty-six races showed, in 135 out of a possible 325 contrasts, six reactions which were interpreted as indicating hermaphroditism, would render the species in the same class with the willows and others of the flowering plants called dioecious. That sexual dimorphism, strictly speaking, does not exist in higher plants is strongly suggested by past observations and experimentation, but the term sexual dimorphism is currently applied to the so-called dioecious condition in forms like the willow, despite the familiar exceptions.

Conclusion no. 2, that the sexual races of *C. echinulata* and *Mucor V* as separated by us are unable to form progametes with any of the twenty-six races of *C. bertholletiae* studied, is too sweeping a statement, and will be shown later to be incorrect. In place of "are unable to form" should have been written "have not been observed to form" progametes.

The statements of fact in conclusions nos. 3, 4, and 7 are what one could make in regard to a heterothallic species. Conclusion no. 5, that a condition of hermaphroditism exists in some strains, seems somewhat opposed to the fact brought out in no. 6, that these hermaphrodites do not themselves take part in conjugation when growing alone in pure cultures, "a fact which indicates that this species is not homothallic," according to BURGER. "Homothallic" it will be remembered is a term used by us to indicate a hermaphroditic condition in gametophytes. The line of reasoning is as follows: some strains are hermaphrodites, in none of the hermaphroditic strains did branches of the same hyphae conjugate, therefore the species is not hermaphroditic.

Earlier in the paper the fact that the stock tubes containing the individual twenty-six races did not produce zygospores under nutrient and temperature conditions favorable for their formation showed according to BURGER "that the cultures were pure and not a mixture of strains." On the contrary, BURGER'S own data show that lack of zygospore formation cannot be a proof of freedom from mixture of strains. Table I makes the matter clear. The minus race no. 4 fails to form zygospores with the plus race no. 10. If nos. 4 and 10 were mixed in a tube culture, therefore, they would not be expected to form zygospores, and yet the plus component (no. 10) of this mixture would form zygospores with the minus races 3, 7, 8, etc., while the minus component (no. 4) would form zygospores with the plus races nos. 9 and 14. The tube containing the mixture suggested would be able to conjugate, therefore, with both plus and minus strains, and such a reaction is BURGER'S proof of hermaphroditism in *Cunninghamella*. Table I shows that eighteen out of the twenty-six races could be mixed to form twenty different combination pairs capable of reacting with both plus and minus races. If all of the 325 possible contrasts had been made between the twenty-six races instead of only the 135 actually attempted, it is probable that a considerably larger number of pairs of races, capable when mixed of producing zygospores with both plus and minus races, would be evident. So far as the data of BURGER go, however, they are sufficient to show that absence of zygospores in a culture cannot be offered as proof that it is not a mixture of strains; and to indicate that infection, if it occurred, rather than the existence of pseudoheterothallic hermaphrodites, might be the cause of zygospores in cultures where they would not be expected on the basis of sexual dimorphism.

BURGER believed he had eliminated the possible objection that his cultures had been mixed by making several single spore cultures from each of the strains nos. 9, 3, and 21, and obtaining zygospores whenever such cultures from one of these strains were contrasted with those from either of the other two strains. The test, on the face of it, may appear to be a critical one, and in fact if only these three strains had been kept in cultivation they would now afford an opportunity of critically retesting data upon which BURGER'S theories are based. Since, however, these cultures were destroyed before the publication of his paper in which their peculiar behavior is described, it will be necessary to depend upon circumstantial rather than upon direct evidence. As seen from table I, nos. 9 and 3 may be considered good plus and minus races, and in consequence should be expected to give reactions when grown together. In consequence, interest centers rather in strain no. 21. This race it will be remembered gave the most aberrant reactions, and together with nos. 14 and 20 is able to account for all the evidence that can be brought forward in support of BURGER'S theory of pseudoheterothallism. No 21 is predominantly minus and so should be expected to give reactions with the plus strain no. 9. The abnormal reaction, therefore, is between nos. 21 and 3. The surprising thing about these tests is that apparently there were no controls. Each of the three single spore cultures of no. 3 were contrasted against each of the four single spore cultures of no. 21, but nothing was said about control contrasts between the subcultures of no. 3, nor of contrasts between the subcultures of no. 21, nor is mention anywhere made of uninoculated controls to discover what the danger might be from air infection of spores of the opposite sexes. For aught we know, single spore subcultures of any race might have appeared to produce zygospores when contrasted together at the time BURGER made his single spore cultures, which was apparently at the end of his series of contrasts with the twenty-six races. Neither in these single spore culture contrasts nor in any of the others is the abundance of zygospores graded. A single zygospore or a limited number which might make the investigator suspicious of mixture of strains in his stock culture or of infection in his contrast culture apparently have been classified as of equal value with our grades *A* and *B*.

In a previous paper (12) attention was called to the peculiar danger of air borne infection of *Cunninghamella* when forms of this genus had previously been grown in the laboratory. *Cunninghamella*, it may be remembered, was first described as an *Oedocephalum*, a hyphomycetous genus with exogenous spores, but was later (2) shown to be a heterothallic mucor by the isolation of its sexual races and their combination to form zygospores. It has already been shown that another investigator who found zygospores in his cultures after planting the mycelia from single spores was apparently misled into a theory of hermaphroditism for *Rhizopus* on account of unsuspected infection of his cultures with sexual races of the same species. It seems reasonable to suspect that BURGER has fallen into a similar error, since he gives no evidence to the contrary, rather than to believe he has discovered a sexual condition unparalleled in the experience of other critical workers.

There are a number of perhaps minor matters in the body of BURGER's paper, such as the use of the terms neutral and zygotactic, to which objection might be made. Enough has been said, however, to indicate that his data do not inevitably lead to his main conclusion of pseudoheterothallic hermaphroditism in *Cunninghamella*.

BURGER'S CONCLUSIONS COMPARED WITH NEW DATA

It will be remembered that of the cultures used by BURGER, *Cunninghamella echinulata* plus and minus, *C. elegans* plus, *Mucor V* plus and minus, and his race no. 21 of *C. bertholletiae* were obtained from us. These races are still running, and it has been possible therefore to compare their behavior with the observations of BURGER on the same material.

The second conclusion in his summary, to the effect that neither the sexual strains of our *C. echinulata* nor those of our *Mucor V* are able to form progametes with any one of twenty-six cultures of *C. bertholletiae*, is contrary to our experience. Table II shows

TABLE II

"IMPERFECT HYBRIDIZATION" BETWEEN RACES OF *Cunninghamella bertholletiae* AND *Mucor V* PLUS AND MINUS; *Mucor V* PLANTED 4:00 P.M., 11/19/19; *C. bertholletiae* PLANTED 11:00 A.M., 11/20/19; RECORDS TAKEN 2:00 TO 4:00 P.M., 11/22/19; NUTRIENT NO. 380 (BURGER'S OATMEAL AGAR); c AND d INDICATE GRADES OF IMPERFECT HYBRIDIZATION; O INDICATES ABSENCE OF OBSERVED REACTION

C. bertholletiae	Plus races						Neutral races		Minus races					
	217	227	268	234	464	456	215	452	266	457	459	213	241	180
Mucor V plus. . .	O	O	O	O	O	O	O	O	c	c	O	d	c	O
Mucor V minus. .	c	d	c	c	c	O	O	O	O	O	O	O	O	O

the results of contrasts between the sexual races of *Mucor V* and testers from the collection of races of *C. bertholletiae* grown on oatmeal agar made up according to BURGER's method of preparation. The majority of the races (including no. 213, which is BURGER's no. 21) showed "imperfect hybridizations" with the opposite sex of *Mucor V*. The nutrient chosen does not appear to be the best for the reaction, but was used to make the conditions of the experiment so far as possible comparable with those reported in the paper under discussion. "Imperfect hybridization" between *Mucor V* plus and our race no. 213 has been obtained on other nutrients, but the reaction with this particular race has never been strong and might readily have been missed had we employed a less successful method of observation (12).

Our old test races of *C. echinulata* (nos. 885 and 886) are able to form "imperfect hybridization" reactions with a number of the races of *C. bertholletiae*, although no sexual reaction between them and our race no. 213 has been observed.

Our race no. 213, which is the same as BURGER'S no. 21 and is the strain which furnished the strongest evidence for his theory of pseudoheterothallic hermaphroditism, has been tested against eighty-eight other races of the same species obtained from Brazil nuts from various localities. In all these tests it has reacted, if at all, only as a minus. In BURGER'S experience, although predominantly a minus, it produced zygospores in three combinations with the seventeen other minus races, a total of 17.5 per cent of the contrasts between it and other minus races. If it had reacted in the same manner we should have expected it to produce zygospores with a minimum of eleven of our sixty-eight other minus races. As a matter of fact, it showed reactions with none of these minus races. That so great a difference really exists between our minus strains and those studied by BURGER seems unlikely.

BURGER seems not to have observed the imperfect sexual reactions between races of *C. bertholletiae* which failed to carry through to zygospore formation. Partly for this reason perhaps, despite his own evidence already adduced to the contrary, he failed to appreciate the fact that absence of zygospore formation in a culture is not a proof of its freedom from mixture with strains of the opposite sex. The imperfect reactions in *C. bertholletiae* will be discussed later. In our experience the plus race no. 465 forms only imperfect reactions with the minus race 457. In consequence, when these two races are planted mixed in a Petri culture in contrast with the plus race no. 217 and the minus race no. 459, a triangular reaction has been obtained, as shown in fig. 1, where the mixture is represented as forming zygospores with *both* the plus race no. 217 and the minus race no. 459, while the latter two are also forming zygospores together. Although we have obtained the triangular reaction shown in fig. 1, which BURGER considered a proof that sexual dimorphism does not exist in *Cunninghamella*, we know in this case that the reaction is due to a mixture of strains and not to pseudoheterothallic hermaphroditism. BURGER'S conclusions, therefore, are not justified from his own data, and the few races which it has been possible to retest from among those studied by him have shown either reactions which he considered impossible or have failed to show the reactions which he found and upon which his theory of pseudoheterothallic hermaphroditism in *Cunninghamella* was based. It must be emphasized, however, that despite the necessity for considering the evidence for sex intergrades in heterothallic mucors open to serious criticism, there is no proof at hand that such intergrades do not exist. A somewhat detailed consideration of the evidence for them in *Cunninghamella* has been given to indicate a few of the dangers into which even one with some experience with cultural methods is likely to fall. The data already published (**10**) and to be presented in the following pages show that sex intergrades must be extremely rare in the mucors, and place the burden of proof on observers who think they have found evidence for their occurrence.

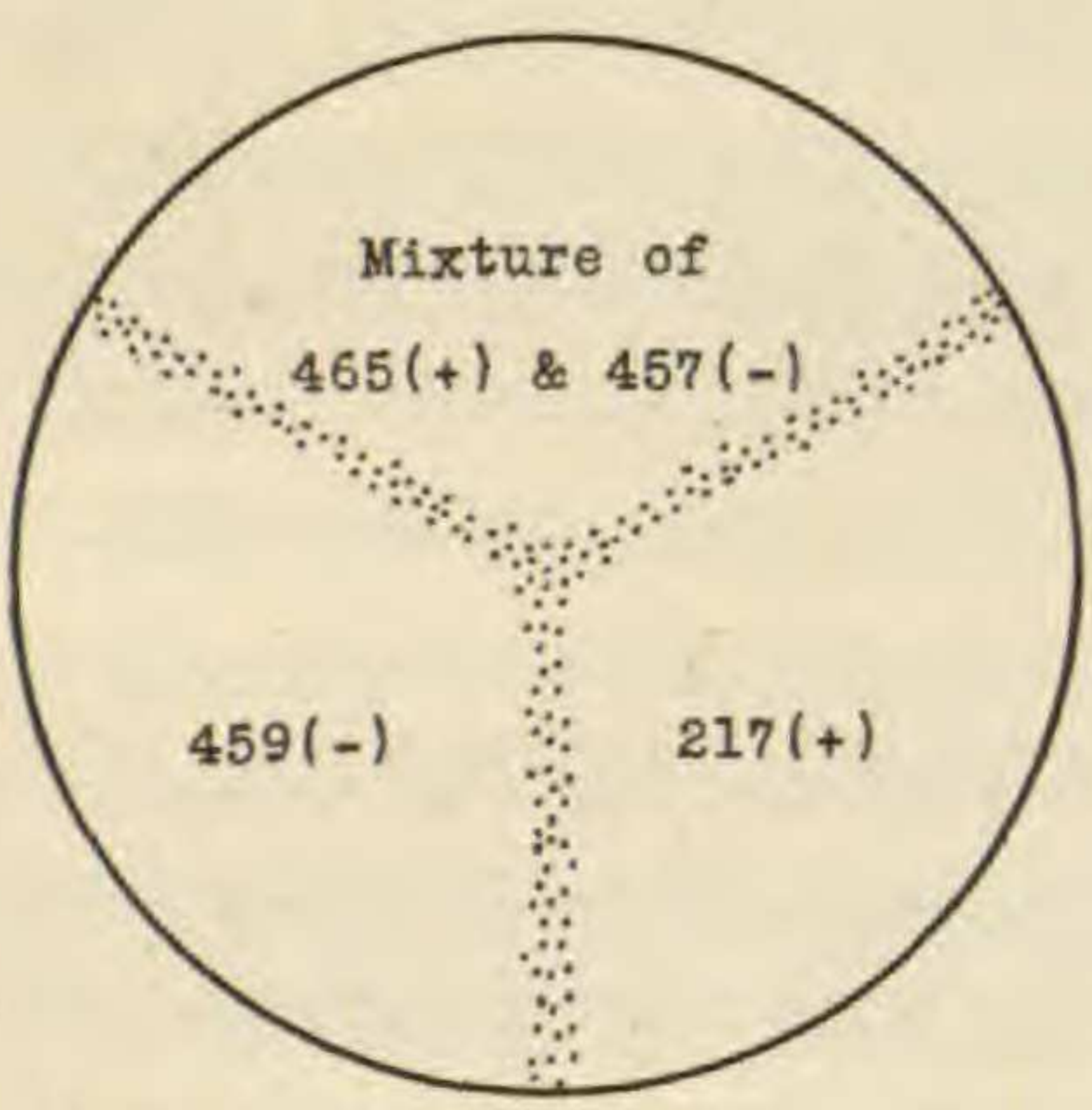


FIG. 1.—Diagram representing Petri dish culture: at lower right and left were planted respectively plus and minus races 217 and 459 which are forming zygospores (represented by dots at line of contact between them); in upper third was planted a mixture of plus and minus races 465 and 457 which fail to form zygospores with each other, but form them with the respective opposite sexes 459 and 217.

New data on Cunninghamella

Tests of the sexual condition in *Cunninghamella* were made with 202 races of four species; forty-two races of *C. elegans*, eighteen races of *C. echinulata*, eighty-nine races of *C. bertholletiae*, and fifty-three races of a species as yet unidentified.[4] The method of running the

[4] The discrepancy between the number of races given here and that listed in a previous publication (**10**) is due to the separation of *Cunninghamella A* from the other species and the omission of four races from the tables on account of infection in the stock tubes, because of incomplete records or for other reasons. All told, including tests with other genera, a considerably larger number of contrasts has been made with *Cunninghamella* than is reported.

gross cultures to obtain the races to be investigated, as well as the detailed methods of making contrasts between them has been described in a previous publication (**12**) and need not be repeated here. Table III gives the origin of the different races used in the tests. Samples of different types of soil were taken from different stations, chiefly near Cold Spring Harbor, and were the source of races of *C. elegans* and *C. echinulata*. Brazil nuts furnished both *C. bertholletiae* and *C. echinulata*, as well as the undetermined species *A*. All the gross cultures were given a serial number preceded by the letter *T* or *H*. The individual nuts in these cultures were indicated by capital letters, and the same was done for the spots on the soil and bread cultures from which transfers were made. In some cases more than a single transfer was made from an individual nut, as is shown by nos. 737 and 738. Generally more than a single race was isolated from each gross culture which showed fruits of the fungus sought, since, as table III shows, sexually distinct races are frequently present in the same gross culture. Undoubtedly among our numbered races some are duplicates, but duplication would probably not have been avoided if only a single race had been taken from each purchased collection of nuts.

More races of a single species were taken from *T*117 than from any other gross culture. From this culture, however, both plus and minus sexes were obtained, and the various races of the same sex are far from all being duplicates, as may be seen by comparing the records of nos. 732, 733, and 739, shown in table VII *A*. Despite the facts that the opposite sexes were frequently found to be present in the same culture and that the gross cultures were run at a temperature favorable for sexual reproduction, no zygospores of *Cunninghamella* in gross culture were found. Their absence may be due to the relatively meager growth of the fungus under the conditions in gross cultures.

The tests with the different species may be considered separately. The individual and mean grades were assigned as already described (**10**, **12**). For the most part, individual contrasts were made only once, since it seemed more profitable to obtain somewhat roughly graded records of a relatively large number of separate

TABLE III

LIST OF RACES OF *Cunninghamella* INVESTIGATED SHOWING RACE NUMBER; SPECIES, WHETHER *C. bertholletiae* (*C. berth.*) *C. echinulata* (*C. ech.*), *C. elegans* (*C. eleg.*), OR THE UNDETERMINED SPECIES *C.A.*; IN CASE OF BRAZIL NUTS, PLACE IN WHICH NUTS WERE PURCHASED IS INDICATED.

Race no.	Species	Culture no.	Substratum	Locality represented	Plus	Neutral	Minus
179..	C. berth.	H1 E	Brazil nuts	Huntington, N.Y.	x	.....	.....
180..	C. berth.	H1 B	Brazil nuts	Huntington, N.Y.	.....	.....	x
181..	C. A.	H1 C	Brazil nuts	Huntington, N.Y.	x	.....	.....
182..	C. A.	H1 E	Brazil nuts	Huntington, N.Y.	.....	.....	x
183..	C. berth.	H1 B	Brazil nuts	Huntington, N.Y.	.....	.....	x
184..	C. A.	H1 G	Brazil nuts	Huntington, N.Y.	.....	.....	x
185..	C. berth.	H1 A	Brazil nuts	Huntington, N.Y.	.....	.....	x
186..	C. berth.	H1 D	Brazil nuts	Huntington, N.Y.	.....	.....	x
187..	C. A.	H2 C	Brazil nuts	Huntington, N.Y.	.....	.....	x
188..	C. A.	H2	Brazil nuts	Huntington, N.Y.	.....	.....	x
189..	C. berth.	H2 B	Brazil nuts	Huntington, N.Y.	.....	.....	x
190..	C. A.	H2 B	Brazil nuts	Huntington, N.Y.	x	.....	.....
191..	C. A.	H2 C	Brazil nuts	Huntington, N.Y.	x	.....	.....
192..	C. berth.	H2 C	Brazil nuts	Huntington, N.Y.	.....	.....	x
193..	C. A.	H2 E	Brazil nuts	Huntington, N.Y.	x	.....	.....
213..	C. berth.	....	....	"Centralstelle," Holland	.....	.....	x
214..	C. berth.	T12 C	Brazil nuts	New York City	.....	.....	x
215..	C. berth.	T13 Y	Brazil nuts	New York City	.....	x	.....
216..	C. berth.	T27 A	Brazil nuts	Huntington, N.Y.	.....	.....	x
217..	C. berth.	T38 A	Brazil nuts	Oyster Bay, N.Y.	x	.....	.....
218..	C. berth.	T38 D	Brazil nuts	Oyster Bay, N.Y.	x	.....	.....
219..	C. berth.	T38 E	Brazil nuts	Oyster Bay, N.Y.	.....	.....	x
220..	C. berth.	T39 A	Brazil nuts	Oyster Bay, N.Y.	.....	.....	x
221..	C. berth.	T39 B	Brazil nuts	Oyster Bay, N.Y.	.....	.....	x
222..	C. berth.	T39 D	Brazil nuts	Oyster Bay, N.Y.	.....	.....	x
223..	C. berth.	T40 A	Brazil nuts	Oyster Bay, N.Y.	.....	.....	x
224..	C. A.	T40 D	Brazil nuts	Oyster Bay, N.Y.	.....	.....	x
225..	C. A.	T40 B	Brazil nuts	Oyster Bay, N.Y.	.....	.....	x
226..	C. berth.	T40 C	Brazil nuts	Oyster Bay, N.Y.	.....	.....	x
227..	C. berth.	T40 E	Brazil nuts	Oyster Bay, N.Y.	x	.....	.....
228..	C. berth.	T48 A	Brazil nuts	Hicksville, N.Y.	.....	.....	x
229..	C. ech.	T13 G	Brazil nuts	New York City	x	.....	.....
232..	C. berth.	T50 F	Paradise nuts	New York City	.....	.....	x
233..	C. berth.	T51 A	Brazil nuts	New York City	.....	.....	x
234..	C. berth.	T51 B	Brazil nuts	New York City	x	.....	.....
245..	C. A.	T51 E	Brazil nuts	New York City	x	.....	.....
236..	C. ech.	T51 H	Brazil nuts	New York City	x	.....	.....
237..	C. berth.	T52 A	Brazil nuts	New York City	.....	.....	x
238..	C. ech.	T52 C	Brazil nuts	New York City	x	.....	.....
239..	C. A.	T52 D	Brazil nuts	New York City	.....	.....	x
240..	C. berth.	T53 B	Brazil nuts	Worcester, Mass.	.....	.....	x
241..	C. berth.	T53 C	Brazil nuts	Worcester, Mass.	.....	.....	x
242..	C. A.	T53 D	Brazil nuts	Worcester, Mass.	x	.....	.....
243..	C. berth.	T54 A	Brazil nuts	Washington, D.C.	.....	.....	x
244..	C. berth.	T54 B	Brazil nuts	Washington, D.C.	.....	.....	x
245..	C. berth.	T55 A	Brazil nuts	Brooklyn, N.Y.	.....	.....	x

TABLE III—*Continued*

Race no.	Species	Culture no.	Substratum	Locality represented	Plus	Neutral	Minus
246..	C. berth.	T55 B	Brazil nuts	Brooklyn, N.Y.	.....	.....	x
247..	C. berth.	T55 F	Brazil nuts	Brooklyn, N.Y.	.....	x	.....
248..	C. ech.	T66 A	Brazil nuts	New York City	x	.....	.....
249..	C. berth.	T66 B	Brazil nuts	New York City	.....	x	.....
250..	C. ech.	T66 C	Brazil nuts	New York City	.....	.....	x
252..	C. berth.	T67 A	Brazil nuts	New York City	.....	.....	x
253..	C. berth.	T67 C	Brazil nuts	New York City	.....	.....	x
254..	C. berth.	T68 A	Brazil nuts	Brooklyn, N.Y.	.....	.....	x
255..	C. ech.	T68 A	Brazil nuts	Brooklyn, N.Y.	x	.....	.....
256..	C. A.	T68 B	Brazil nuts	Brooklyn, N.Y.	.....	.....	x
257..	C. A.	T68 C	Brazil nuts	Brooklyn, N.Y.	.....	.....	x
258..	C. berth.	T68 D	Brazil nuts	Brooklyn, N.Y.	.....	.....	x
259..	C. A.	T68 E	Brazil nuts	Brooklyn, N.Y.	x	.....	.....
260..	C. A.	T68 E	Brazil nuts	Brooklyn, N.Y.	x	.....	.....
261..	C. A.	T73 A	Brazil nuts	New York City	x	.....	.....
262..	C. berth.	T73 B	Brazil nuts	New York City	.....	.....	x
265..	C. ech.	T44 B	Soil	Cold Spring Harbor	.....	.....	x
266..	C. berth.	T50 F	Paradise nuts	New York City	.....	.....	x
267..	C. A.	T52 D	Brazil nuts	New York City	x	.....	.....
268..	C. berth.	T52 D	Brazil nuts	New York City	x	.....	.....
269..	C. A.	T68 C	Brazil nuts	Brooklyn, N.Y.	.....	.....	x
270..	C. A.	T27 B	Brazil nuts	Huntington, N.Y.	.....	.....	x
271..	C. berth.	T38 B	Brazil nuts	Oyster Bay, N.Y.	x	.....	.....
272..	C. A.	T68 D	Brazil nuts	Brooklyn, N.Y.	.....	.....	x
273..	C. A.	T73 C	Brazil nuts	New York City	x	.....	.....
274..	C. berth.	T73 C	Brazil nuts	New York City	.....	.....	x
275..	C. berth.	T74 E	Brazil nuts	New York City	.....	.....	x
372..	C. berth.	T76 B	Brazil nuts	New York City	.....	.....	x
373..	C. berth.	T76 C	Brazil nuts	New York City	.....	x	.....
446..	C. berth.	....	Brazil nuts	Brooklyn, N.Y.	.....	.....	x
447..	C. berth.	T74 C	Brazil nuts	New York City	.....	.....	x
448..	C. berth.	T75 A	Brazil nuts	New York City	.....	.....	x
449..	C. berth.	T79	Brazil nuts	New York City	.....	.....	x
450..	C. berth.	T80 B	Brazil nuts	New York City	.....	x	.....
451..	C. berth.	T80 E	Brazil nuts	New York City	.....	.....	x
452..	C. berth.	T81 B	Brazil nuts	Brooklyn, N.Y.	.....	x	.....
453..	C. berth.	T81 E	Brazil nuts	Brooklyn, N.Y.	.....	.....	x
454..	C. berth.	T96 A	Brazil nuts	Storrs, Conn.	.....	.....	x
455..	C. A.	T96 E	Brazil nuts	Storrs, Conn.	.....	.....	x
456..	C. berth.	T96 C	Brazil nuts	Storrs, Conn.	x	.....	.....
457..	C. berth.	T97 B	Brazil nuts	Amsterdam, N.Y.	.....	.....	x
458..	C. berth.	T99 A	Brazil nuts	Amsterdam, N.Y.	.....	.....	x
459..	C. berth.	T99 D	Brazil nuts	Amsterdam, N.Y.	.....	.....	x
460..	C. berth.	T99 C	Brazil nuts	Amsterdam, N.Y.	.....	.....	x
461..	C. berth.	T100 A	Brazil nuts	Amsterdam, N.Y.	.....	.....	x
462..	C. berth.	T101 A	Brazil nuts	Amsterdam, N.Y.	.....	.....	x
463..	C. berth.	T101 B	Brazil nuts	Amsterdam, N.Y.	.....	.....	x
464..	C. berth.	T101 C	Brazil nuts	Amsterdam, N.Y.	x	.....	.....
465..	C. berth.	T98	Brazil nuts	Amsterdam, N.Y.	x	.....	.....
466..	C. eleg.	T29 D	Soil	Cold Spring Harbor	x	.....	.....
467..	C. eleg.	T36 B	Soil	Cold Spring Harbor	.....	x	.....
468..	C. eleg.	T36 C	Soil	Cold Spring Harbor	.....	.....	x
469..	C. eleg.	T44 D	Soil	Cold Spring Harbor	x	.....	.....
470..	C. eleg.	T58 D	Soil	Cold Spring Harbor	x	.....	.....

TABLE III—*Continued*

Race no.	Species	Culture no.	Substratum	Locality represented	Plus	Neutral	Minus
471..	C. eleg.	T60 B	Soil	Cold Spring Harbor	x	.....	.....
472..	C. eleg.	T60 D	Soil	Cold Spring Harbor	.....	.....	x
473..	C. eleg.	T60 E	Soil	Cold Spring Harbor	x	.....	.....
474..	C. eleg.	T61 D	Soil	Cold Spring Harbor	x	.....	.....
475..	C. eleg.	T61 C	Soil	Cold Spring Harbor	.....	.....	x
476..	C. eleg.	T61 E	Soil	Cold Spring Harbor	.....	.....	x
477..	C. eleg.	T61 G	Soil	Cold Spring Harbor	.....	.....	x
478..	C. eleg.	T62 A	Soil	Cold Spring Harbor	.....	.....	x
479..	C. eleg.	T62 A	Soil	Cold Spring Harbor	.....	.....	x
480..	C. eleg.	T63 A	Soil	Cold Spring Harbor	.....	.....	x
481..	C. eleg.	T64 D	Soil	Cold Spring Harbor	x	.....	.....
482..	C. eleg.	T64 C	Soil	Cold Spring Harbor	x	.....	.....
483..	C. eleg.	T64 E	Soil	Cold Spring Harbor	.....	.....	x
484..	C. eleg.	T64 F	Soil	Cold Spring Harbor	x	.....	.....
485..	C. eleg.	T65 C	Soil	Cold Spring Harbor	x	.....	.....
486..	C. eleg.	T65 A	Soil	Cold Spring Harbor	.....	.....	x
487..	C. eleg.	T84 A	Soil	Cold Spring Harbor	x	.....	.....
488..	C. eleg.	T84 B	Soil	Cold Spring Harbor	.....	.....	x
489..	C. eleg.	T84 D	Soil	Cold Spring Harbor	.....	.....	x
490..	C. eleg.	T85 B	Soil	Cold Spring Harbor	.....	.....	x
491..	C. eleg.	T85 D	Soil	Cold Spring Harbor	.....	.....	x
492..	C. eleg.	T85 E	Soil	Cold Spring Harbor	x	.....	.....
493..	C. eleg.	T86 A	Soil	Cold Spring Harbor	x	.....	.....
494..	C. eleg.	T86 C	Soil	Cold Spring Harbor	x	.....	.....
495..	C. eleg.	T86 E	Soil	Cold Spring Harbor	x	.....	.....
496..	C. eleg.	T86 F	Soil	Cold Spring Harbor	x	.....	.....
497..	C. eleg.	T86 G	Soil	Cold Spring Harbor	x	.....	.....
498..	C. eleg.	T87 B	Soil	Cold Spring Harbor	x	.....	.....
499..	C. eleg.	T87 F	Soil	Cold Spring Harbor	x	.....	.....
500..	C. eleg.	T89 A	Soil	Cold Spring Harbor	x	.....	.....
501..	C. eleg.	T89 B	Soil	Cold Spring Harbor	x	.....	.....
502..	C. eleg.	T89 C	Soil	Cold Spring Harbor	x	.....	.....
503..	C. eleg.	T92 B	Soil	Cold Spring Harbor	x	.....	.....
504..	C. eleg.	T92 D	Soil	Cold Spring Harbor	x	.....	.....
505..	C. eleg.	T92 D	Soil	Cold Spring Harbor	x	.....	.....
506..	C. eleg.	T92 E	Soil	Cold Spring Harbor	.....	.....	x
507..	C. eleg.	T92 G	Soil	Cold Spring Harbor	.....	.....	x
508..	C. A.	T52	Brazil nuts	New York City	x	.....	.....
510..	C. ech.	....	Laboratory infection	Washington, D.C.	.....	.....	x
511..	C. A.	T73 D	Brazil nuts	New York City	.....	.....	x
512..	C. A.	T75 A	Brazil nuts	New York City	x	.....	.....
513..	C. A.	T75 B	Brazil nuts	New York City	.....	.....	x
514..	C. A.	T75 C	Brazil nuts	New York City	.....	.....	x
515..	C. A.	T75 F	Brazil nuts	New York City	x	.....	.....
516..	C. A.	T76 A	Brazil nuts	New York City	.....	.....	x
517..	C. A.	T76 D	Brazil nuts	New York City	.....	.....	x
518..	C. A.	T76 E	Brazil nuts	New York City	.....	.....	x
519..	C. A.	T79 B	Brazil nuts	New York City	.....	.....	x
520..	C. A.	T79 F	Brazil nuts	New York City	.....	.....	x
521..	C. A.	T80 A	Brazil nuts	New York City	x	.....	.....
522..	C. A.	T81 C	Brazil nuts	Brooklyn, N.Y.	x	.....	.....
523..	C. A.	T81 G	Brazil nuts	Brooklyn, N.Y.	.....	.....	x
524..	C. A.	T96 E	Brazil nuts	Storrs, Conn.	x	.....	.....

TABLE III—*Continued*

Race no.	Species	Culture no.	Substratum	Locality represented	Plus	Neutral	Minus
525..	C. ech.	T98 C	Brazil nuts	Amsterdam, N.Y.	x	.....	.....
526..	C. ech.	T99 A	Brazil nuts	Amsterdam, N.Y.	.....	.....	x
527..	C. ech.	T97 A	Brazil nuts	Amsterdam, N.Y.	x	.....	.....
528..	C. ech.	T99 E	Brazil nuts	Amsterdam, N.Y.	x	.....	.....
529..	C. ech.	T99 E	Brazil nuts	Amsterdam, N.Y.	.....	.....	x
718..	C. berth.	T111 E	Brazil nuts	Norway, Me.	.....	.....	x
719..	C. berth.	T112 F	Brazil nuts	Parkersburg, W.Va.	.....	.....	x
720..	C. berth.	T113 C	Brazil nuts	Louisville, Ky.	.....	x	.....
721..	C. berth.	T114 A	Brazil nuts	Franklin, Ind.	.....	.....	x
722..	C. berth.	T114 D	Brazil nuts	Franklin, Ind.	.....	.....	x
723..	C. berth.	T115 A	Brazil nuts	Hickory, N.C.	.....	x	.....
724..	C. berth.	T115 B	Brazil nuts	Hickory, N.C.	.....	.....	x
725..	C. berth.	T115 B	Brazil nuts	Hickory, N.C.	.....	.....	x
726..	C. berth.	T115 F	Brazil nuts	Hickory, N.C.	.....	.....	x
727..	C. berth.	T116 B	Brazil nuts	Knoxville, Tenn.	.....	.....	x
728..	C. berth.	T116 C	Brazil nuts	Knoxville, Tenn.	.....	.....	x
729..	C. berth.	T116 G	Brazil nuts	Knoxville, Tenn.	.....	.....	x
730..	C. berth.	T117 B	Brazil nuts	Knoxville, Tenn.	.....	.....	x
731..	C. berth.	T117 B	Brazil nuts	Knoxville, Tenn.	.....	.....	x
732..	C. berth.	T117 B	Brazil nuts	Knoxville, Tenn.	.....	.....	x
733..	C. berth.	T117 B	Brazil nuts	Knoxville, Tenn.	.....	.....	x
734..	C. berth.	T117 C	Brazil nuts	Knoxville, Tenn.	.....	.....	x
735..	C. berth.	T117 C	Brazil nuts	Knoxville, Tenn.	.....	.....	x
736..	C. berth.	T117 D	Brazil nuts	Knoxville, Tenn.	.....	.....	x
737..	C. berth.	T117 D	Brazil nuts	Knoxville, Tenn.	.....	.....	x
738..	C. berth.	T117 D	Brazil nuts	Knoxville, Tenn.	x	.....	.....
739..	C. berth.	T117 E	Brazil nuts	Knoxville, Tenn.	.....	.....	x
740..	C. berth.	T118 A	Brazil nuts	Knoxville, Tenn.	.....	.....	x
741..	C. berth.	T118 D	Brazil nuts	Knoxville, Tenn.	.....	.....	x
742..	C. A.	T111 A	Brazil nuts	Norway, Me.	.....	.....	x
743..	C. A.	T111 B	Brazil nuts	Norway, Me.	.....	.....	x
744..	C. A.	T111 C	Brazil nuts	Norway, Me.	.....	.....	x
745..	C. A.	T111 G	Brazil nuts	Norway, Me.	.....	x	.....
747..	C. ech.	T113 B	Brazil nuts	Louisville, Ky.	x	.....	.....
748..	C. A.	T113 B	Brazil nuts	Louisville, Ky.	x	.....	.....
749..	C. A.	T113 B	Brazil nuts	Louisville, Ky.	x	.....	.....
750..	C. ech.	T113 C	Brazil nuts	Louisville, Ky.	.....	.....	x
751..	C. A.	T113 D	Brazil nuts	Louisville, Ky.	.....	.....	x
752..	C. A.	T113 D	Brazil nuts	Louisville, Ky.	x	.....	.....
753..	C. A.	T116 A	Brazil nuts	Knoxville, Tenn.	.....	.....	x
754..	C. A.	T116 D	Brazil nuts	Knoxville, Tenn.	.....	x	.....
755..	C. A.	T116 D	Brazil nuts	Knoxville, Tenn.	x	.....	.....
756..	C. ech.	T116 E	Brazil nuts	Knoxville, Tenn.	.....	.....	x
757..	C. A.	T116 E	Brazil nuts	Knoxville, Tenn.	.....	.....	x
758..	C. A.	T116 H	Brazil nuts	Knoxville, Tenn.	.....	.....	x
759..	C. A.	T118 D	Brazil nuts	Knoxville, Tenn.	x	.....	.....
779..	C. berth.	T115 D	Brazil nuts	Hickory, N.C.	x	.....	.....
885..	C. ech.	....	Laboratory culture	Cambridge, Mass.	x	.....	.....
886..	C. ech.	....	Laboratory culture	Cambridge, Mass.	.....	.....	x

contrasts than to attempt to secure more accurate records by averaging the grades of a relatively few contrasts which had been several times repeated. If any of the cultures had become infected or in any other way appeared abnormal, the contrasts of course were repeated. In a few cases, especially in the earlier contrasts with *C. bertholletiae*, zygospores were found where, on the basis of a strict sexual dimorphism, they would not be expected. A repetition of these contrasts under improved technique gave the results incorporated in table VII A, and indicated that their earlier aberrant behavior was due to infection with the opposite sexes of the same species. All contrasts with species of *Cunninghamella* have been grown in the incubating oven at 24°–27°C.

Cunninghamella elegans

Table IV shows the tests with *C. elegans*. Twelve races were used as testers, and in all 426 contrast combinations were made with the total forty-two races. Of these, twenty-five were plus, sixteen were minus, and one, on account of its failure to show reactions in any of the combinations tested, has provisionally been listed as a neutral.

Cunninghamella echinulata

Table V shows the tests with *C. echinulata*. All the 153 possible contrast combinations were made with the total eighteen races. Of these, ten were plus, eight were minus, and none failed to show a sexual reaction in at least two contrast combinations. Since no reactions occurred when races with like sign were contrasted together, only the contrasts between plus and minus races are represented in the table.

Cunninghamella A

Table VI shows the tests with the undetermined species of *Cunninghamella* provisionally termed *Cunninghamella A*. It is a form intermediate in appearance between *C. bertholletiae* and *C. echinulata*, and was at first confused with them. In tube cultures it approaches more nearly the habit and color of *C. echinulata*. From this species, however, it may readily be distinguished microscopically, especially by the lack of conspicuous echinulations on the conidia. The form, however, needs a more careful study than

TABLE IV

SUMMARY OF TESTS OF *Cunninghamella elegans:* RELATIVE STRENGTH OF ZYGOSPORE FORMATION IN DIFFERENT COMBINATIONS INDICATED BY LETTERS A TO D; ABSENCE OF ZYGOSPORES INDICATED BY O; GRADES ASSIGNED TO INDIVIDUAL RACES ARE MEANS OF THEIR REACTIONS WITH TESTERS OF OPPOSITE SEX; NO. 230 NUTRIENT USED, CONSISTING OF 2 PER CENT AGAR, 2 PER CENT DRY MALT EXTRACT, 2 PER CENT DEXTROSE, AND 0.1 PER CENT MEAT PEPTONE.

Grade	Races	Minus testers					Plus testers						
		475	472	468	478	507	496	466	474	469	470	471	473
	Plus races												
2.80	496	B	B	B	B	C	....	O	O	O	O	O	O
2.60	482	A	B	B	C	D	O	O	O	O	O	O	O
2.40	487	B	A	B	D	D	O	O	O	O	O	O	O
2.40	505	C	B	B	D	B	O	O	O	O	O	O	O
2.20	466	B	C	D	C	B	O	....	O	O	O	O	O
2.20	474	A	A	C	D	O	O	O	....	O	O	O	O
2.20	494	A	B	C	D	D	O	O	O	O	O	O	O
2.20	499	B	B	B	C	O	O	O	O	O	O	O	O
2.20	504	B	B	C	D	C	O	O	O	O	O	O	O
2.00	469	C	B	C	B	O	O	O	O	....	O	O	O
2.00	500	B	C	B	D	D	O	O	O	O	O	O	O
2.00	502	C	C	C	D	B	O	O	O	O	O	O	O
1.80	471	C	B	B	D	O	O	O	O	O	O	....	O
1.80	497	C	C	C	C	D	O	O	O	O	O	O	O
1.60	470	C	C	B	D	O	O	O	O	O	....	O	O
1.60	484	B	C	D	D	D	O	O	O	O	O	O	O
1.60	498	C	C	C	C	O	O	O	O	O	O	O	O
1.40	492	D	O	B	D	C	O	O	O	O	O	O	O
1.40	473	D	D	D	C	C	O	O	O	O	O	O	....
1.40	481	C	C	C	D	O	O	O	O	O	O	O	O
1.40	485	C	C	C	D	O	O	O	O	O	O	O	O
1.40	493	C	C	D	D	D	O	O	O	O	O	O	O
1.40	495	C	C	D	D	D	O	O	O	O	O	O	O
1.40	503	C	D	D	D	C	O	O	O	O	O	O	O
1.00	501	O	O	C	C	D	O	O	O	O	O	O	O
	Neutral races												
0.00	467	O	O	O	O	O	O	O	O	O	O	O	O
	Minus races												
2.71	506	O	O	O	O	O	B	C	B	B	B	B	C
2.57	472	O	....	O	O	O	B	C	A	B	C	B	D
2.57	476	O	O	O	O	O	B	B	B	B	C	B	D
2.57	480	O	O	O	O	O	B	B	C	C	A	B	D
2.43	475	....	O	O	O	O	B	B	A	C	C	C	D
2.29	479	O	O	O	O	O	C	B	C	C	C	B	C
2.29	489	O	O	O	O	O	B	B	C	C	C	C	C
2.14	468	O	O	....	O	O	B	D	C	C	B	B	D
2.14	488	O	O	O	O	O	B	C	B	C	C	C	D
2.14	490	O	O	O	O	O	B	C	C	C	C	C	C
1.86	478	O	O	O	....	O	B	C	D	B	D	D	C
1.86	483	O	O	O	O	O	A	C	D	C	D	C	D
1.71	491	O	O	O	O	O	C	C	C	C	C	D	D
1.71	486	O	O	O	O	O	B	C	C	C	C	O	D
1.00	507	O	O	O	O	....	C	B	O	O	O	O	C
0.71	477	O	O	O	O	O	O	D	D	D	D	D	O
Grades (all combinations)		2.36	2.24	2.12	1.44	1.12	2.69	2.25	2.12	2.06	1.94	1.94	1.31

it has received before it can justly be described as a distinct species. The manner in which the races reacted in combinations first suggested that another species was included in the collections, and a later inspection and microscopic examination showed that species

TABLE V

SUMMARY OF TESTS OF *Cunninghamella echinulata:* RELATIVE STRENGTH OF ZYGOSPORE FORMATION IN DIFFERENT COMBINATIONS INDICATED BY LETTERS A TO D; ABSENCE OF ZYGOSPORES INDICATED BY O; GRADES ASSIGNED TO INDIVIDUAL RACES ARE MEANS OF THEIR REACTIONS WITH TESTERS OF OPPOSITE SEX; CONTRASTS BETWEEN RACES OF SAME SEX MADE BUT NOT REPRESENTED; IN ALL CASES THEY FAILED TO PRODUCE ZYGOSPORES; NO. 362 NUTRIENT USED, CONSISTING OF 2 PER CENT AGAR, 2 PER CENT WHEY POWDER, AND 1 PER CENT DEXTROSE.

Grade	Minus races	Grade									
		2.50	2.25	1.87	1.63	1.63	1.50	1.25	1.25	1.13	0.50
		Plus races									
		747	885	527	229	525	238	236	255	248	528
2.70	886..........	B	B	B	B	B	C	B	B	C	C
2.30	265..........	C	C	C	C	B	C	C	B	B	C
2.00	750..........	C	B	B	A	O	B	B	D	D	O
1.60	510..........	B	B	B	C	C	C	O	D	O	O
1.50	529..........	B	C	C	C	O	C	C	O	C	O
1.10	526..........	C	B	O	O	B	O	O	C	D	O
0.70	756..........	C	C	C	O	O	D	O	O	O	O
0.50	250..........	B	O	O	O	C	O	O	O	O	O

A could be distinguished from the other species. Six races were used as testers, and in all 297 contrast combinations were made with the total fifty-three races. Of these, twenty-two were plus, twenty-nine were minus, and two, on account of their failure to show reactions in any of the combinations tested, were listed as neutrals. Imperfect sexual reactions, indicated by small letters in table VI, will be discussed under the following species.

CUNNINGHAMELLA BERTHOLLETIAE

Table VII *A* shows the tests with *C. bertholletiae*. Fifteen races were used as testers, and in all 1215 combinations were made with the total eighty-nine races. Of these, twelve were plus, sixty-nine were minus, and eight, on account of their failure to show reactions

TABLE VI

SUMMARY OF TESTS OF *Cunninghamella A:* RELATIVE STRENGTH OF ZYGOSPORE FORMATIONS IN DIFFERENT COMBINATIONS INDICATED BY CAPITAL LETTERS A TO D; STRENGTH OF IMPERFECT REACTIONS BY SMALL LETTERS a TO d; ABSENCE OF SEXUAL REACTIONS INDICATED BY O; GRADES ASSIGNED TO INDIVIDUAL RACES ARE MEANS OF THEIR REACTIONS WITH TESTERS OF OPPOSITE SEX; NO. 362 NUTRIENT USED, CONSISTING OF 2 PER CENT AGAR, 2 PER CENT WHEY POWDER, AND 1 PER CENT DEXTROSE.

Grade	Races	Minus testers			Plus testers		
		269	182	257	515	242	181
	Plus races						
4.00	515	A	A	A	....	O	O
3.67	242	A	A	B	O	....	O
3.33	181	B	B	A	O	O	....
3.00	191	B	B	B	O	O	O
2.67	759	C	B	B	O	O	O
2.33	267	C	C	B	O	O	O
2.33	748	C	B	C	O	O	O
2.00	261	C	C	C	O	O	O
2.00	755	C	C	C	O	O	O
1.67	235	C	C	D	O	O	O
1.67	259	C	C	D	O	O	O
1.67	508	C	D	C	O	O	O
1.67	521	D	C	C	O	O	O
1.67	749	D	C	C	O	O	O
1.33	260	C	D	D	O	O	O
1.33	273	C	D	D	O	O	O
1.33	524	D	C	D	O	O	O
1.00	190	D	D	D	O	O	O
1.00	512	D	O	C	O	O	O
0.67	522	D	D	O	O	O	O
0.33	752	D	O	O	O	O	O
0.33	193	D	O	O	O	O	O
	Neutral races						
0.00	745	O	O	O	O	O	O
0.00	754	O	O	O	O	O	O
	Minus races						
3.67	182	O	......	O	A	A	B
3.67	257	O	O	....	A	B	A
3.67	269	....	O	O	A	A	B
3.33	517	O	O	O	A	b	b
3.33	523	O	O	O	A	b	b
3.00	744	O	O	O	A	C	B
3.00	455	O	O	O	A	C	B
2.33	514	O	O	O	A	D	C
2.33	256	O	O	O	B	C	C
1.67	224	O	O	O	B	D	D
1.67	188	O	O	O	d	b	D
1.33	513	O	O	O	B	O	D
1.00	239	O	O	O	B	O	O
1.00	751	O	O	O	b	O	O
0.67	184	O	O	O	C	O	O
0.67	270	O	O	O	C	O	O

TABLE VI—*Continued*

Grade	Races	Minus testers			Plus testers		
		269	182	257	515	242	181
	Minus races						
0.67	272	O	O	O	C	O	O
0.67	511	O	O	O	C	O	O
0.67	516	O	O	O	C	O	O
0.67	518	O	O	O	C	O	O
0.67	520	O	O	O	C	O	O
0.67	742	O	O	O	C	O	O
0.67	743	O	O	O	C	O	O
0.67	753	O	O	O	C	O	O
0.67	758	O	O	O	C	O	O
0.67	757	O	O	O	c	O	O
0.67	187	O	O	O	c	O	O
0.33	225	O	O	O	D	O	O
0.33	519	O	O	O	O	O	d
Grades (all combinations)		1.91	1.86	1.82	2.59	0.97	1.03

in any of the combinations tested, have been provisionally listed as neutral.

C. bertholletiae seems to differ from the other species of *Cunninghamella* investigated except species *A*, and in fact from all the other mucors which have been studied in the same manner, in that between certain races imperfect sexual reactions have been found which do not lead to zygospore formation. It is possible that such reactions may occur more frequently than is realized. In contrasting the first few testers of a given species, the practice has been to look for imperfect reactions at an early stage of development, and, if none are found, to examine the culture dishes in later series only at the end of the growth period when imperfect reactions would not readily be recognized. It is thus possible that some of the zero records for the contrasts of species *A* in table VI would be replaced by grades of imperfect sexual reaction if they had all been retested and inspected at an early growth period. Imperfect reactions are graded in the tables by small letters instead of by the capitals used for zygospore formation. The reaction might readily be confused with the early stages of zygospore formation or the final stages of "imperfect hybridization." Imperfect hybridization has heretofore been found to occur only between the opposite sexes of different species. When dealing with races of

TABLE VII

SUMMARY OF TESTS OF *Cunninghamella bertholletiae:* RELATIVE STRENGTH OF ZYGOSPORE FORMATION IN DIFFERENT COMBINATIONS INDICATED BY CAPITAL LETTERS A TO D, RELATIVE STRENGTH IN IMPERFECT SEXUAL REACTION INDICATED BY SMALL LETTERS a TO d; PRODUCTION OF PARTHENOSPORES (*a*-ZYGOSPORES) INDICATED BY CAPITAL LETTER FOLLOWED BY AN ASTERISK; ABSENCE OF OBSERVED SEXUAL REACTION INDICATED BY O; GRADES ASSIGNED TO INDIVIDUAL RACES ARE MEANS OF THEIR SEXUAL REACTIONS WITH TESTERS OF OPPOSITE SEX; NO. 230 NUTRIENT USED, CONSISTING OF 2 PER CENT AGAR, 2 PER CENT DRY MALT EXTRACT, 2 PER CENT DEXTROSE, AND 0.1 PER CENT MEAT PEPTONE.

Grade	Races	*A*. Intraspecific reactions															*B*. Reactions with species A				
		Minus testers							Neutral testers		Plus testers						Minus testers			Plus testers	
		266	457	459	213	183	241	180	215	452	217	227	268	234	464	456	188	455	269	191	515
	Plus races																				
3.00	217.......	A	B	B	B	b	b	C	O	O	...	O	O	O	O	O	d	c	C*	O	...
2.71	234.......	b	b	c	b	c	b	b	O	O	O	O	O	...	O	O	b	d	C*	O	O
2.71	268.......	B	A	B	B	c	c	c	O	O	O	O	...	O	O	O	c	b	C*	O	...
2.57	465.......	C	b	B	b	c	b	C	O	O	O	O	O	O	O	O	...	...	D*	O	...
2.57	227.......	B	C	B	B	c	b	c	O	O	O	...	O	O	O	O	c	...	C*	O	...
2.43	464.......	B	B	B	B	c	d	C	O	O	O	O	O	O	...	O	c	...	D*	O	...
2.14	738.......	B	C	B	A	D	O	c	O	O	O	O	O	O	O	O	...	...	...	O	...
1.71	179.......	b	c	D	d	b	C	O	O	O	O	O	O	O	O	O	...	...	C*	O	...
1.43	456.......	C	b	c	D	d	O	d	O	O	O	O	O	O	O	...	O	...	...	O	...
1.29	218.......	D	c	c	O	C	C	O	O	O	O	O	O	O	O	O	...	...	O	O	...
1.29	271.......	c	C	c	d	c	O	O	O	O	O	O	O	O	O	O	...	...	C*	O	O
0.43	779.......	D	O	D	O	O	D	O	O	O	O	O	O	O	O	O	...	...	...	O	...
	Neutral races																				
0.00	215.......	O	O	O	O	O	O	O	...	O	O	O	O	O	O	O	O	...	...	O	...
0.00	247.......	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	...	...	...	O	...
0.00	249.......	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	...	...	...	O	...
0.00	373.......	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	...	...	...	O	...
0.00	450.......	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	...	...	...	O	...
0.00	452.......	O	O	O	O	O	O	O	O	...	O	O	O	O	O	O	O	...	...	O	...
0.00	720.......	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	...	...	...	O	...
0.00	723.......	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	...	...	...	O	...
	Minus races																				
3.00	732.......	O	O	O	O	O	O	O	O	O	A	A	B	C	B	c	...	...	O	b	C*
3.00	266.......	...	O	O	O	O	O	O	O	O	A	B	B	b	B	C	O	O	...	d	...
3.00	274.......	O	O	O	O	O	O	O	O	O	B	B	B	b	A	c	...	...	O	c	...
3.00	729.......	O	O	O	O	O	O	O	O	O	A	A	A	O	A	c	...	...	O	c	...
3.00	457.......	O	...	O	O	O	O	O	O	O	B	C	A	b	B	b	O	O	...	c	...
2.83	737.......	O	O	O	O	O	O	O	O	O	A	B	B	c	B	c	...	...	...	c	...
2.67	213.......	O	O	O	...	O	O	O	O	O	B	B	B	b	B	D	O	...	O	c	...
2.67	252.......	O	O	O	O	O	O	O	O	O	B	B	B	c	B	c	...	...	...	b	...
2.67	262.......	O	O	O	O	O	O	O	O	O	A	B	B	b	B	O	...	...	...	O	...
2.67	459.......	O	O	...	O	O	O	O	O	O	B	B	B	c	B	c	O	...	O	c	...
2.50	731.......	O	O	O	O	O	O	O	O	O	B	B	B	C	B	d	...	...	...	a	D*
2.50	736.......	O	O	O	O	O	O	O	O	O	A	A	B	O	C	c	...	...	...	b	...
2.33	458.......	O	O	O	O	O	O	O	O	O	C	C	C	b	B	c	...	...	...	b	...
2.17	219.......	O	O	O	O	O	O	O	O	O	B	D	C	a	B	O	...	...	...	D	...
2.00	180.......	O	O	O	O	O	O	...	O	O	C	c	c	b	C	d	O	...	O	O	...
2.00	183.......	O	O	O	O	...	O	O	O	O	b	c	c	c	c	d	O	O	O	b	...

TABLE VII—*Continued*

Grade	Races	*A.* Intraspecific reactions															*B.* Reactions with species A				
		Minus testers							Neutral testers		Plus testers						Minus testers			Plus testers	
		266	457	459	213	183	241	180	215	452	217	227	268	234	464	456	188	455	269	191	515
	Minus races																				
2.00	241	O	O	O	O	O	...	O	O	O	b	b	c	b	d	O	O	O	O	c	...
1.67	186	O	O	O	O	O	O	O	O	O	b	c	c	b	O	O	...	...	...	O	...
1.67	244	O	O	O	O	O	O	O	O	O	b	b	c	c	O	O	...	...	...	b	...
1.50	192	O	O	O	O	O	O	O	O	O	d	c	c	c	c	O	...	...	...	O	...
1.50	216	O	O	O	O	O	O	O	O	O	b	c	c	c	O	O	...	...	...	O	...
1.50	223	O	O	O	O	O	O	O	O	O	b	c	c	c	O	O	...	...	...	c	...
1.50	226	O	O	O	O	O	O	O	O	O	c	c	c	c	d	O	...	...	...	O	...
1.50	232	O	O	O	O	O	O	O	O	O	c	c	c	O	B	O	...	...	...	c	...
1.50	233	O	O	O	O	O	O	O	O	O	b	c	c	c	O	O	...	...	...	O	...
1.50	451	O	O	O	O	O	O	O	O	O	b	c	c	O	O	c	...	...	...	O	...
1.33	221	O	O	O	O	O	O	O	O	O	c	c	c	c	O	O	...	...	...	d	...
1.33	258	O	O	O	O	O	O	O	O	O	C	c	c	d	D	O	...	...	...	O	...
1.33	448	O	O	O	O	O	O	O	O	O	d	c	c	d	O	c	...	...	...	b	...
1.33	463	O	O	O	O	O	O	O	O	O	b	c	c	d	O	O	...	...	...	O	...
1.33	733	O	O	O	O	O	O	O	O	O	c	O	d	d	c	c	...	...	...	b	...
1.17	185	O	O	O	O	O	O	O	O	O	D	c	c	d	d	O	...	...	...	b	...
1.17	189	O	O	O	O	O	O	O	O	O	d	c	c	c	O	O	...	...	...	O	...
1.17	222	O	O	O	O	O	O	O	O	O	d	c	c	c	O	O	...	...	...	c	...
1.17	243	O	O	O	O	O	O	O	O	O	c	c	c	d	O	O	...	...	...	O	...
1.17	275	O	O	O	O	O	O	O	O	O	c	c	c	d	O	O	...	...	...	b	...
1.17	460	O	O	O	O	O	O	O	O	O	B	c	c	O	O	O	...	...	...	c	...
1.17	461	O	O	O	O	O	O	O	O	O	c	c	c	d	O	O	...	...	...	O	...
1.17	718	O	O	O	O	O	O	O	O	O	B	d	O	O	C	d	...	...	...	O	...
1.00	220	O	O	O	O	O	O	O	O	O	c	c	c	O	O	O	...	...	...	O	...
1.00	228	O	O	O	O	O	O	O	O	O	c	c	c	O	O	O	...	...	...	O	...
1.00	254	O	O	O	O	O	O	O	O	O	c	c	c	O	O	O	...	...	...	O	...
1.00	446	O	O	O	O	O	O	O	O	O	d	c	c	d	O	O	...	...	...	a	...
1.00	449	O	O	O	O	O	O	O	O	O	d	c	c	d	O	O	...	...	...	c	...
0.83	214	O	O	O	O	O	O	O	O	O	d	c	c	O	O	O	...	...	...	c	...
0.83	240	O	O	O	O	O	O	O	O	O	d	c	c	O	O	O	...	...	...	c	...
0.83	246	O	O	O	O	O	O	O	O	O	d	c	c	O	O	O	...	...	...	O	...
0.83	447	O	O	O	O	O	O	O	O	O	d	c	c	O	O	O	...	...	...	c	...
0.83	453	O	O	O	O	O	O	O	O	O	d	c	c	O	O	O	...	...	...	c	...
0.83	454	O	O	O	O	O	O	O	O	O	d	c	c	O	O	O	...	...	...	O	...
0.83	462	O	O	O	O	O	O	O	O	O	d	c	c	O	O	O	...	...	...	O	...
0.83	741	O	O	O	O	O	O	O	O	O	c	d	O	O	O	C	...	...	...	d	...
0.83	372	O	O	O	O	O	O	O	O	O	d	O	O	a	O	O	...	...	...	O	...
0.67	253	O	O	O	O	O	O	O	O	O	c	c	O	O	O	O	...	...	...	b	...
0.67	722	O	O	O	O	O	O	O	O	O	d	O	O	O	b	O	...	...	...	c	...
0.67	726	O	O	O	O	O	O	O	O	O	c	O	O	O	c	O	...	...	...	c	...
0.67	730	O	O	O	O	O	O	O	O	O	c	O	d	O	d	O	...	...	...	O	...
0.67	735	O	O	O	O	O	O	O	O	O	B	O	O	O	d	O	...	...	...	d	...
0.67	740	O	O	O	O	O	O	O	O	O	c	O	O	O	c	O	...	...	...	c	...
0.50	721	O	O	O	O	O	O	O	O	O	d	O	O	O	O	C	...	...	...	c	...
0.50	734	O	O	O	O	O	O	O	O	O	c	O	O	O	d	O	...	...	...	c	...
0.33	237	O	O	O	O	O	O	O	O	O	O	O	c	O	O	O	...	...	...	O	...
0.33	245	O	O	O	O	O	O	O	O	O	d	d	O	O	O	O	...	...	...	O	...

TABLE VII—*Continued*

Grade	Races	*A*. Intraspecific reactions															*B*. Reactions with species A				
		Minus testers							Neutral testers		Plus testers						Minus testers			Plus testers	
		266	457	459	213	183	241	180	215	450	217	227	268	234	464	456	188	455	269	191	515
	Minus races																				
0.33	719.......	O	O	O	O	O	O	O	O	O	c	O	O	O	O	O	...	...	...	c	...
0.33	724.......	O	O	O	O	O	O	O	O	O	d	O	O	O	d	O	...	...	...	c	...
0.33	727.......	O	O	O	O	O	O	O	O	O	c	O	O	O	O	O	...	...	...	c	...
0.33	728.......	O	O	O	O	O	O	O	O	O	d	O	O	O	d	O	...	...	...	a	...
0.33	739.......	O	O	O	O	O	O	O	O	O	O	O	O	O	c	O	...	...	...	O	...
0.17	725.......	O	O	O	O	O	O	O	O	O	d	O	O	O	O	O	...	...	...	c	...
Grades (all combinations)		2.50	2.42	2.33	2.08	1.83	1.67	1.33	0.00	0.00	2.09	1.71	1.68	1.09	1.07	0.52					

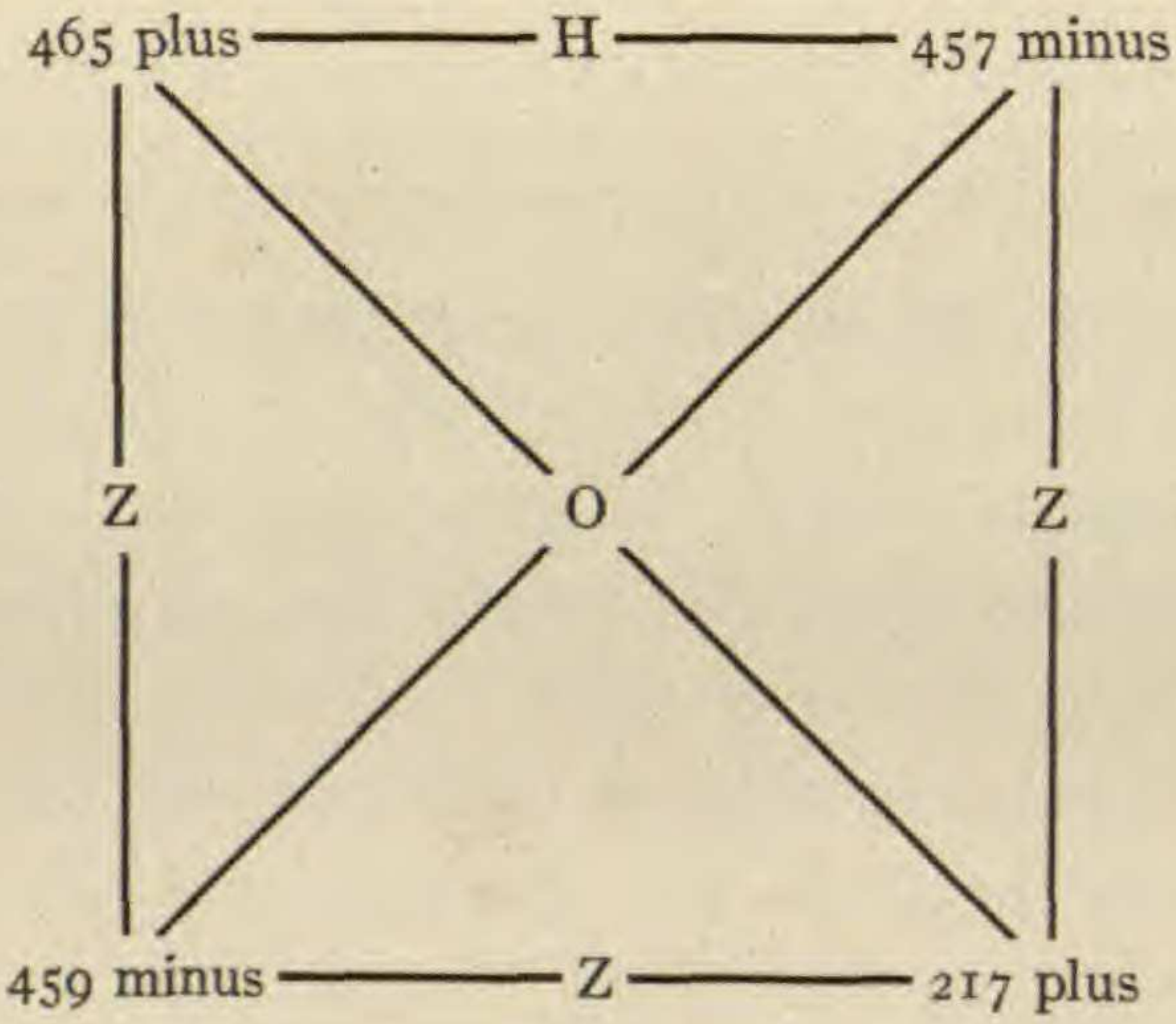
the same species, if any reaction was initiated at all, it was carried through to the production of zygospores, except of course for some obviously detrimental check in environmental conditions. The sexual process resulting in the production of zygospores may be considered the sum of two distinct reactions; first, the formation of opposed progametes or at most gametes; second, the dissolution of the cross walls between the gametes and the growth of the fusion cell into a zygospore. Only the first reaction can take place when the plus and minus races contrasted belong to different species.[5]

A number of facts indicate, however, that in the races listed under *C. bertholletiae* we are dealing with a single species. The essential uniformity of the strains in morphological appearance speaks for specific identity, and the production of zygospores fails to separate them into any consistent groups. A quadrangular reaction within selected groups of four may be discerned from table VII *A*. Thus the same four races shown in fig. 1 form the following quadrangle, in which *Z* stands for zygospore formation, *H* for imperfect sexual reaction, and *O* for no sexual reaction. Other similar quadrangles may be assembled from table VII *A*. The fact that only imperfect reactions are found in certain contrasts when

[5] True hybrids have been reported between closely related species of *Mucor* by Saito and Naganishi (19).

zygospores would be expected does not alter the sex of the races involved. When they take part in any sexual reactions at all, they are consistently either plus or minus. The first reaction of the sexual process is sufficient to indicate their sex, and gives an index of their sexual vigor. In calculating the mean grades of sexual activity for the different races, therefore, it has been considered fairest to give the imperfect reactions equal weight with zygospore formation.

What are the causes which prevent one combination in a quadrangular reaction from carrying the sexual process through to completion is a question requiring further study. In certain cases,



at least, the distance between the inoculations of opposing strains seems to be a matter of some importance. In *Circinella spinosa* it has always been necessary to inoculate the opposite sexes very close together in order to obtain zygospores, which are not formed beyond a few millimeters from the points of inoculation. In a few cases a retest of an imperfect reaction between races of *C. bertholletiae*, but with inoculations close together, has shown zygospore formation.

Certain contrasts which were repeated with inoculations at the usual distance apart gave different reactions from those first obtained, as may be seen by a comparison of table VII *A* with table VIII where the retest contrasts are listed. In table VIII there is, as might be expected, a certain amount of change in the grades assigned to the strength of the reactions. The present interest, whoever, centers upon the grades inclosed in parentheses, which indicate reactions which have changed from a perfect to an imperfect reaction as shown by the production of stages resembling imperfect hybrids in place of zygospores. It will be seen that there are certain unexplained irregularities in the production of zygospores or of only imperfect reactions which indicate that the preliminary tests have not discovered all the factors involved. Enough has been learned, it is believed, to indicate that some of the factors are environmental which determine whether a sexual process in this species goes through to

TABLE VIII

RETESTS OF CONTRASTS BETWEEN RACES OF *Cunninghamella bertholletiae:* CAPITAL LETTERS INDICATE GRADES OF ZYGOSPORE FORMATION; SMALL LETTERS INDICATE GRADES OF IMPERFECT SEXUAL REACTION; LETTERS INCLOSED IN PARENTHESES SHOW CHANGE IN TYPE OF REACTION FROM ZYGOSPORE FORMATION TO IMPERFECT REACTIONS; REACTIONS WITH ASTERISK INDICATE PRESENCE OF PARTHENOSPORES; NO. 230 NUTRIENT USED.

Races	266	729	732	457	737	213	459	731	219	180	241	232	460	718	741	721
217			A					A		C		c	(O)	C		
234			(b)		c			(a)		c						
268										c			c			
465	C			b		b	B									
227									B							
179	c						C				(d)*					
456	C	c		c		C									(c)*	(c)*
218											C					
271	c			(c)												

completion with the formation of zygospores or is confined to the first reaction with the formation of progametes or at the most gametes.

Although environmental differences not readily controlled in the cultures may have some influence upon the extent of the sexual reaction, the genetic constitution of the individual races in the main must be responsible for their sexual behavior. We have not succeeded, however, in an attempt to subject the genetic differences to a factorial interpretation. Distinct classes of plus and minus races differing sharply in the strength of their sexual activity or in their capacity to form zygospores or only imperfect reactions with certain other races do not seem to exist. Thus certain contrasts from table VII *A* may be arranged in such a way that no fewer than five differences in reaction are shown both in the plus and minus strains chosen to form table IX. A graded series is indicated which might indefinitely be expanded as more and more races were tested.

"Imperfect hybridization" between species

Tables IV to VII *A* deal with sexual reactions between races within the individual species concerned. In tables II, VII *B*, X, and XI are given the results of contrasting individual races of one species with those of another species. Many of the contrasts were made before the species *Cunninghamella A* was separated from

TABLE IX

Arrangement of selected races from table VII *A* showing graded differences in strength of reaction when contrasted: capital letters indicate grades of zygospores, small letters grades of imperfect sexual reaction.

Minus races	Plus races				
	217	227	456	779	234
266	A	B	C	D	b
459	B	B	c	D	c
213	B	B	D	O	b
457	B	C	b	O	b
180	C	c	d	O	b

C. echinulata and *C. bertholletiae*. Those between the testers *H* and *D* and the races of *Cunninghamella A* were made merely for the purpose of identifying the sex of the latter, and were not graded, since they were not originally intended for publication. It has seemed best, however, to include these and the reactions in table VII *B*, since they furnish cumulative evidence in regard to sexual dimorphism in *Cunninghamella*. Two races of *C. elegans* (nos. 496 and 506, respectively plus and minus) failed to show reactions with the old plus and minus testers of *C. echinulata* (nos. 885 and 886). In table VII *B* certain combinations are starred because in them the imperfect hybridization reactions led to the production of parthenospores (*a*-zygospores). When certain races of high sexual vigor are contrasted, gametes which have been formed but which have been unable to unite may develop into thick-walled sculptured spores, which are with difficulty distinguished from the true zygospores. Superficial inspection under low magnifications would undoubtedly lead to their classification as zygospores, but it is not unlikely that in our records, especially the earlier ones on *C. bertholletiae*, contrasts may have been listed as weak zygospore reactions,

TABLE X

SUMMARY OF REACTIONS BETWEEN DIFFERENT SPECIES OF *Cunninghamella:* Z INDICATES ZYGOSPORES; SMALL LETTERS INDICATE GRADED IMPERFECT REACTIONS

	C. bertholletiae		C. echinulata		C. elegans	
	217 plus	266 minus	885 plus	886 minus	496 plus	506 minus
C. bertholletiae						
217 plus	....	Z	O	c	O	c
266 minus	Z	....	c	O	c	O
C. echinulata						
885 plus	O	c	....	Z	O	O
886 minus	c	O	Z	....	O	O
C. elegans						
496 plus	O	c	O	O	....	Z
506 minus	c	O	O	O	Z	....

TABLE XI

Cunninghamella A: UNGRADED "IMPERFECT HYBRIDIZATION" REACTIONS WITH PLUS AND MINUS MUCOR TESTERS *H* AND *D; H* IN BODY OF TABLE INDICATES IMPERFECT REACTIONS

Mucor	Plus races						Minus races						
	515	242	759	260	273	522	182	269	188	751	270	511	225
H plus	O	O	O	O	O	O	H	H	H	H	H	H	H
D minus	H	H	H	H	H	H	O	O	O	O	O	O	O

when they should have been called imperfect reactions with formation of parthenospores. A close examination, especially in the younger stages, will show that parthenospores develop from single gametes, and that the suspensor on only one side has a typical appearance, with what appears to be the suspensor on the opposite side frequently more or less rounded off and not closely adnate to the spore. The parthenospores themselves are often distinctly misshapen, but when the zygospores are small, as is true of those of species of *Cunninghamella,* it may be difficult to distinguish them even with careful inspection. Parthenospores have been obtained between certain strong races of different species in other genera with larger zygospores when no doubt of their true nature was likely to occur after a careful examination. Figures of parthenospores formed on homothallic species, at stimulus of contact with a sexually vigorous race of a heterothallic species, are given in an earlier publication (**9**, *pl. I*). The possible presence of parthenospores must not be overlooked in judging reports (**19**) of true hybridization between different species in the mucors.

So far as the reactions between different species of *Cunninghamella* have been tested, they argue for the sexual dimorphism of this genus.

Discussion

The data in the present paper refer only to the mucor genus *Cunninghamella*. A preliminary summary has already been given of tests with other genera (**10**), and it is hoped to publish a detailed account of these tests at a later date. The data so far accumulated show no behavior inconsistent with the idea of a strict sexual dimorphism. The work, especially with *Cunninghamella*, indicates that sex intergrades must be extremely rare if ever present in these forms, despite the fact that they would be expected on a priori grounds and the fact that other observers have thought they had found them.

In the species of *Cunninghamella* there is apparent a graded series so far as the strength of sexual activity is concerned, ranging from a reaction with grade *A* between sexually strong races to grade *O* between sexually weak races. Races which have shown no reactions in any contrast tested are provisionally listed as "neutral." The term neutral is obviously relative, and not meant to indicate absolute absence of sex. The number of races listed as neutral for a given collection tends to decrease as more testers are used in contrasts. Thus it is evident from table VII *A* that if strain no. 217 had not been used as a tester, strains nos. 719, 727, and 725 would have been listed as neutrals rather than as minus strains, since they would have shown no reaction against any of the plus or minus testers used. Neutrals seem to form the low extreme of a continuously graded series of sexual vigor, and the term as applied undoubtedly includes both plus and minus races.

It is doubtful whether much significance can be attributed to the proportion of plus and minus races in the collections of the different species of *Cunninghamella* as indicative of their relative distribution in nature. In *C. bertholletiae* the minus sex seems to greatly predominate over the plus. In *C. elegans* the condition is reversed. The first species was obtained from Brazil nuts bought in different stores, mostly in or around New York City. Many of the gross cultures, therefore, may have originated from the same wholesale shipments. The races may be representative of the shipments from which they came rather than of the locality where they were grown. Experience with *Rhizopus* (4) indicates that in a mixed culture which is producing zygospores in abundance, one is likely to isolate almost exclusively one or the other of the two sexes. The cargo carriers from which the nuts originated may have been infected chiefly with minus strains. That there is considerable diversity in sexual vigor of these strains, however, is seen from the tables. *C. elegans* was obtained from different types of soil around Cold Spring Harbor, and it is possible that collections from other regions would show a predominance of the opposite sex.

The clearest result from the study of *Cunninghamella* is the fact that in 2091 contrasts (2250 including contrasts between different species of *Cunninghamella*) made between 202 races from four different species (see footnote 4) there were none which, if they showed any sexual response at all, reacted otherwise than as either a plus or a minus.

Miss Alice M. Pricket, Miss Margaret Conover, and Miss Mary E. Drummond have assisted in the progress of the investigation which is here reported.

Summary

1. The terms heterothallic and homothallic are distinguished as applied to gametophytic sexual differentiation in the mucors.

2. Types of the evidence in support of sex intergrades in heterothallic mucors are given and criticized.

3. Burger's paper on *Cunninghamella*, in which he concludes that sexual dimorphism does not exist in this genus, is discussed (1) from the standpoint of his own data, (2) from the standpoint of our experience, and the decision is reached that his conclusion is not warranted.

4. Data on *Cunninghamella elegans*, *Cunninghamella A* (an undetermined species), *C. echinulata*, and *C. bertholletiae* give a total of 2250 contrasts between a total of 202 races.

5. In *C. bertholletiae* certain contrast combinations lead to imperfect sexual reactions when zygospores might be expected.

6. In none of the species were races found which reacted as sex intergrades.

7. It is concluded that so far as the material investigated is concerned *Cunninghamella* is sexually dimorphic.

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